Laboratory Rotation Report

Particle-tracking microrheology of cancer cells responding to membrane-targeted molecular self-assembly

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Date of Submission: December 2017
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Aims

This project has implemented the fluorescent particle-tracking technique [Wirtz, 2009] for monitoring the alteration of actin cytoskeleton induced by cancer cell membrane targeted molecular self-assembly.

Background

The life cycle of cell is strongly connected with mechanical deformability of the cytoplasm. For example, processes such as organelles' movement within cytoplasm are partially determined by the frictional forces, and characterize by cytoplasmic viscoelastic properties. Changes in mechanical properties often indicate cell's disease state. For example, in the model of mouse progeria cells are more softer than wild-type [Lammerding et al., 2004]. This affects cells' ability to resist shear forces and allow them migrate more easily in the tissue. Cytoplasm viscoelastic properties mediated by cytoskeleton. Cytoskeleton is one of the targets for cancer treatment. It is formed by three different types of filaments. One of them is actin filament, that is mostly located nearby cellular membrane and in charge of cells motility and shape. Lipid rafts are one of the membrane mesoscale domain that affects actin filaments mesh. Ruthenium (Ru)-complex-based peptidic molecule (Yp) selectively targets cancer cells through lipid rafts [Li, 2017]. This chemical self-assembles into nanofibrils on cervical cancer cells' membrane leading to the contradictory movement of the cell, causing mechanical stress that ruptures the cell.

Mechanical properties could be characterized by such parameters as elastic modulus (G_1), viscous modulus (G_2) and shear viscosity (η). This viscoelastic properties could be measured without applying external forces to the cell, by tracking the movement of embedded beads inside the cytoplasm[Wirtz, 2009] . That technique is called passive microrheology.

If in a liquid, submicron beads perform Brownian motion, that could be described with time-average MSD: $< r^2 >= 4Dt$
If in a solid, each time the bead is driven by thermal energy in random direction, the force pushes it back. So MSD of the bead is constant. $< r^2 >= const.$

In the case of liquid, the elastic modulus is zero $G_1 = 0$, and viscous modulus is proportional to shear viscosity and frequency($G_2 \propto \eta\omega$). While in an elastic solid the viscous modulus is zero $G_2 = 0$, but an elastic modulus doesn't dependent on time $G_1(t) = const$ [Wirtz, 2009].

Analyzes of MSD, viscous and shear modulus as functions of time ($< r^2 >= 4dt^{\alpha}$) illustrate how the movement of embedded bead can indicate mechanical properties of the material whether it is in a liquid state or an elastic solid[Wirtz, 2009].

In order to investigate and quantitively measure the influence of Yp on cells mechanical properties microrheology technique is implemented.

Methods

Cell Culture

Hella cell line was propagated in a cell incubator at 37°C under 5% CO_2. Cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics.

Electroporation

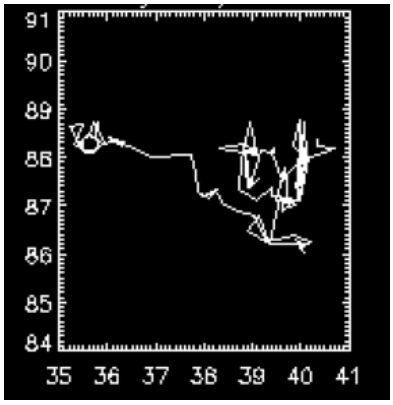
Carboxylate-modified yellow-green fluorescent spheres with about 100nm in diamete (FluoSpheres) were introduced into cells with ECM 399 Electroparation System. The 40 to 120nm pores are formed with this machine. Cells concentration was 2-5 10^6 cells/ml.

Imaging

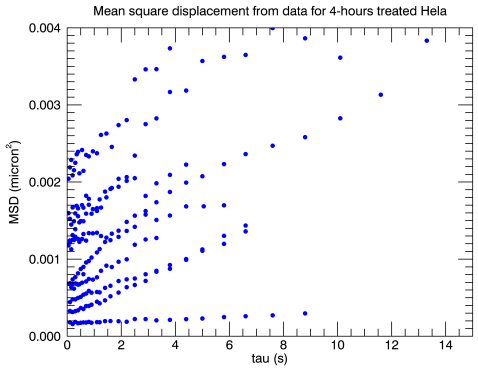
Culture dishes were viewed with confocal microscope(LSM, Carl Zeiss). The beads' dynamics is studied by recording 20s video with 10 frames per second. Particle motion is tracked and analyzed with special written Microrheology routine in IDL (http://www.physics.emory.edu/faculty/weeks//idl/rheo.html).

Results

Time dependent coordinates of the beads are transformed into mean squared displacement, as presented on the Figure 1 .



(a) Trajectory of the bead



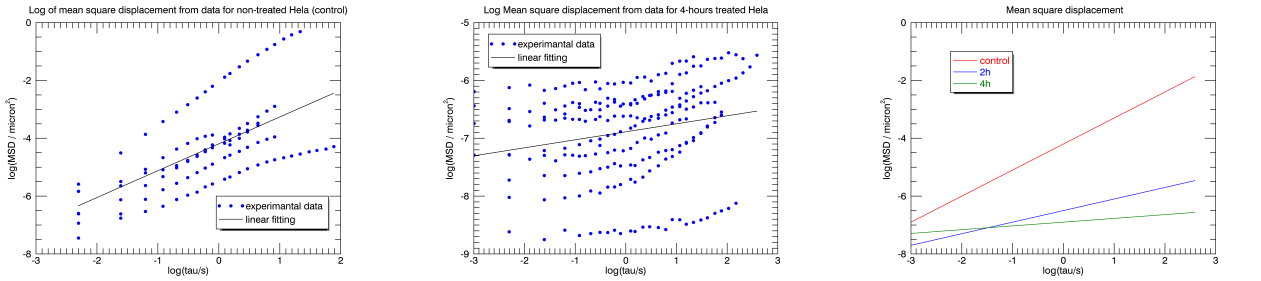
(b) Lag time dependent Mean squared displacement

Figure 1: Analyzes of the movement of the spheres embedded in the cytoplasm

experiment	α	D, $\mu m^2/s$	$\eta, Pa \cdot s$
untreated	0.9 ± 0.1	$(3.7 \pm 0.4)10^{-3}$	(0.6 ± 0.1)
2hours treated	0.4 ± 0.1	$(3.7 \pm 0.4)10^{-4}$	(5.6 ± 0.6)
4hours treated	0.1 ± 0.1	$(2.5 \pm 0.3)10^{-4}$	(8.2 ± 1.0)

Table 1: Shear viscosity and diffusion coefficient by analysis of MSD equation

Before the treatment particles displays high mobility inside the cell, while after the treatment the motion is limited. The MSD in treated and untreared cells shows that untreated Hela cells more viscous rather than elastic, while 4 hours treatment makes the cytoplasm more solid like, decreasing the α from 0.9 ± 0.1 in control to 0.1 ± 0.1 in 4 hours treated cells. Beads of untreated Hela exhibits almost Brownian motion as indicates α. The size of beads is larger than the average mesh size of the cytosceleton network(40-100nm). Sponteneous movements of actin filaments around beads induce displacement of the bead. The shear viscosity of control Hela is (0.6 ± 0.1) Pa s, that is almost twice as smaller than the viscosity of fibroblasts' cytoplasm under the same conditions[Wirtz, 2009]. It confirms that cancer cells are softer than the normal one.

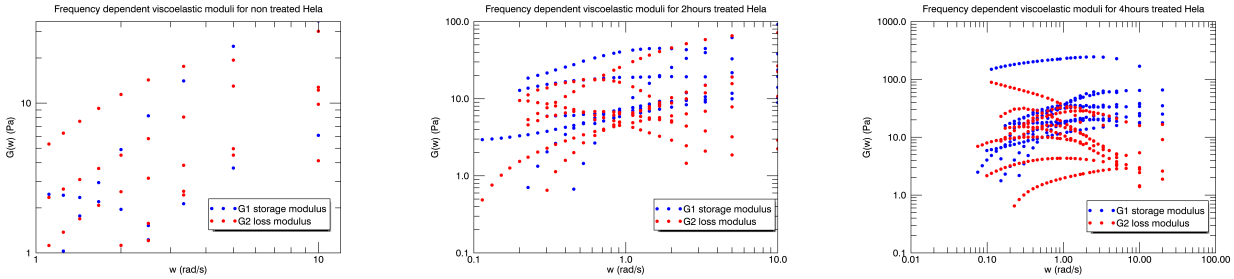


(a) Control (b) 4hours treatmnent (c) Linear approximation of log(MSD)

Figure 2: Logarithm of mean square displacement of the spheres embedded in the cytoplasm

The assumption was that the Yp treatment destruct the cell's cortex making the cytoplasm less tough, decreasing its viscosity. But the result, that was found, is completely opposite. The reason could be the destruction of the actin network led to the conglomerates formation and beads are spatially constrained in actin conglomerates' microdomaines. F-actin staining showed that conglomerates of actin is distributed around the cytoplasm. Yp affect dissassembling of actin filaments and provoke them to create actin conglomerates. Though the viscosity of the cytoplasm increases almost 15 times after 4 hours treatment.

The time-lag dependency of MSD of beads is transformed into frequency-dependent viscoelastic moduli, G_1 and G_2, Figure 3.
On the Figure 3c at long time scale (small ω) $G_2 > G_1$, that indicates that cytoplasm is mostly viscous , but at short timescale $G_2 < G_1$ shows that it is predominantly elastic. While before the treatment (Figure 3a) $G_2 \propto \omega$ indicates that cytoplasm behave mostly viscous
[Wirtz, 2009]



(a) Control (b) 2hours treatmnent (c) 4hours treatment

Figure 3: Frequency-dependent storage(or elastic) modulus and loss(or viscous) modulus of cytoplasm measured by the random displacement of fluorescent spheres embedded in the cell

References

[Lammerding et al., 2004] Lammerding, J., Schulze, P. C., Takahashi, T., Kozlov, S., Sullivan, T., Kamm, R. D., Stewart, C. L., and Lee, R. T. (2004). Lamin a/c deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest*, 113(3):370–8.

[Li, 2017] Li, G. (2017). Patching of lipid rafts by molecular self- assembled nanofibrils suppresses cancer cell migration. *Chem*, 2(2):283–298.

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